

Cytotaxonomy and Cyto geography of *Vicia* sect. *Vicilla* in Northeast of China

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Abstract *Vicia amurensis* has pollen grain with swollen mesocipium, terete style, abaxially sprouting stylar hairs and globes stigma, differing from other five species, *V. amoena*, *V. japonica*, *V. pseudo-orbus*, *V. ramuliflora*, *V. umjunga* and *V. venosa*, in sect. *Vicilla* of Kupicha's System. This result further support taxonomic treatment *V. amurensis* proposed by Endo and Ohashi. The chromosome numbers of 64 populations and karyotypes of 15 populations for sect. *Vicilla* and sect. *Amurensis* were observed. *V. amurensis* and *V. pseudo-orbus* have similar karyotype features such as the same karyotype symmetry, comparable chromosome size and the same type of Sat-chromosomes, and therefore *V. amurensis* in sect. *Amurensis* is assumed to be closely related to *V. japonica* in sect. *Vicilla*. The primary study of nuclear DNA amount was carried out on nine populations of six taxa in sect. *Vicilla* in China. It is primarily discussed that evolutionary trend of karyotype, nuclear DNA amount in sect. *Vicilla* and geographic distribution of different ploidy cytotypes.

Key words: *Vicia* sect. *Vicilla*, Sect. *Amurensis*, Karyotype, Nuclear DNA content, Cyto geography, Cytotaxonomy

Introduction

Kupicha has made a revision of *Vicia* L. in the world since 1976. Sect. *Vicilla* includes about fifteen species. The most taxa in this section belong to Asian species, which distribute in Siberia in Russia, Northeast, North and West of China, Mongolia, Japan and Korea, such as *V. amoena*, *V. amurensis* and *V. japonica* etc.. Three taxa among them, *V. pisiformis*, *V. dumetorum* and *V. sylvatica* mainly distribute in Europe. *V. sylvatica* also distributes in the North of Asia. Chromosome numbers and karyotypes of Asian sect. *Vicilla* were reported by Huiziwara and Kodon (1968) etc..

In genus *Vicia*, a astonishing variation in chromosome size among species is reflected in nuclear DNA amount (Maxted et al., 1991; Raina, 1988, 1990; Narayan et al. 1983). Raina (1990) found that the divergence and evolution of species was accompanied by a seven fold variation in the amount of chromosomal DNA, ranging from 3.85 pg in *V. monantha* to 27.07 pg in *V. faba*. Maxted et al. (1991) and Raina (1990) all observed that the distribution of DNA amount in *Vicia* species. It seemed to be independent from taxonomic position. Recently, Raina et al. (1994) observed that direction of DNA changing in polyploids was species specific and rapid DNA loss to the adaptive advantage in the establishment of colchitetraploids in *Phlox drummondii*, which is in agreement with the conclusions reached by Kenton et al. (1990), and Raina and Rees (1983 a, b). It is proposed that recent or ancient origin of polyploidy be addressed by measuring nuclear DNA amount. This rela-

tion has been observed in *Triticum* (Pegington and Rees, 1970), *Hordeum* (Bennet and Smith, 1971), *Bulnesia* (Poggio and Hunziker, 1986) and *Larrea* (Poggio et al, 1989).

In this paper, karyotype and nuclear DNA content of sect. *Vicilla* and *V. amurensis* of sect. *Amurensis* have been studied. Cytotaxonomic relationship between those sections, evolutionary trend of karyotype and nuclear DNA amount in sect. *Vicilla* and the pattern of geographic distribution of infraspecific tetraploid cytotypes have also been discussed.

Materials and Methods

Plant materials

The seeds of species in this test were collected from wild populations in Northeast of China, Japan and Korea. And some seeds were supplied by the ILDIS Coordination Center, University of Southampton, England (Table 1).

Observation with SEM

Inflorescences of six *Vicia* species, from plants cultivated in Botanical Garden of Harbin Normal University, were fixed in FAA. For observation with SEM, flower organs were obtained from flowers in FAA and dehydrated by an ethanol series. Then the ethanol was removed by isoamylacetate. The materials were dried in a critical point dryer with carbon dioxide and coated with gold. Styles were cut at the middle region to observe the

transfer section. The specimens were observed under a Hitachi S-520 SEM.

Chromosome counts and karyotypes

The seeds were put on a moist filter, and germinated at 22°C. When roots grew and elongated about 1.2 cm length, root tips were cut off and pretreated with saturated β -dichlorobenzene solution for 4-6 h. They were fixed in Carnoy's solution for 3-24 h. Root tips were hydrolyzed in 1Mol/L HCl at 60°C for 6-8 min, stained in modified Carbol fuchin and squashed in 45% acetic acid. Photographs of metaphase cells were taken by

OLYMPUS BH2 microscope. For karyotype analysis, the systems proposed by Leaven et al. (1964) and Stebbins (1971) were followed. Satellite bodies were included in total length. The karyotype symmetry index (As. I. %) was calculated using the formula described by Arano and Saito (1980).

Determination of DNA amount

The seeds of the taxa were directly collected from wild population in Northeast China (Table 1). *Allium cepa* cv. *Alisa Craig* with a known 4C DNA amount of 67.00 pg was used as the measurements standard.

Table 1. Chromosome numbers and origins of *Vicia* sect. *Vicilla* and sect. *Amurensis* in present study

Taxa	2n	Origin
<i>V. amoena</i>	24	Harbin, Li 90020; Zhaodong, Li and Liu 90030; Qiqihaer, Li 90022; Jiagedaqi, Li and Liu 90030; Tahe, Li 90025; Ta yuan, Li and Liu, 90018; Siliji, Li and Liu 90019; Heihe, Li 90024; Tieli Li 90014; Mudanjiang, Liu 90015; Suifenhe, Li and Liu 90017; Mishan, Liu 90019; Hailaer, Li 90033; Baicheng, Li 91009; Baihe, Li and Liu 91015; Jianping, Li 91020; Qianshan, Li 91018, Senyang, Li 91023; Simeng, Li 90033, 90050; Mangui, Li 90039
<i>V. amoena</i> var. <i>Sertcea</i>	12	Daqingguo, Li 91100; Genqika, Li 91103; Yihuta, Li 91101
<i>V. amoena</i> var. <i>oblongifolia</i>	24	Zhaodong, Liu 89001.
<i>V. bifolia</i>	12	Honshus, Saitama Pref. Endo 166, Japan.
<i>V. dumetorum</i>	14	Roy 740600, Romania.
<i>V. fauriei</i>	12	Honshus, Yamagata Pref. Endo 487, Japan.
<i>V. japonica</i>	24	Gulian, Li 90053; Maoershan, Li 90057; Baishilazi, Pei 89059.
<i>V. nipponica</i>	12	Honshus, Miyagi Pref. Endo 589, Japan.
<i>V. pistiformis</i>	12	Roy 740600, Romania.
<i>V. pseudo-orbus</i>	12	Jiagedaqi, Li 90045; Tahe, Li 90046; Silinji, Li and Liu 90048; Qianshan, Li 91046
<i>V. ramuliflora</i>	12	Gulian, Li 91041; Tahe, Li 91042; Silinji, Li 91043; Dailing, Li and Liu 91047; Qianshan, Li 91046; Mudanjiang, Liu 91047; Fenghuangshan, Li 91048; Huangnihe, Liu 91049.
	24	Changbaishan, Li and Liu 91045.
<i>V. niniuga</i>	12	Qiling, Pei 89000; simeng, Li 90043; Jiagedaqi, Li 91001; Tahe, Li 89003.
	24	Yichun, Li 91050; Mudanjiang, Liu 89030; Qianshan, Li 91047; Fenghuangshan, Li 91048; Changbaishan, Li 95006; Choi 95003, Korea.
<i>V. venosa</i>	12	Huangnihe, Liu 90060; Dailing, Liu and Li 90062
<i>V. venosa</i> var. <i>cuspidata</i>	12	Choi 95001, 95002, Korea.
<i>V. amurensis</i>	12	Jiagedaqi, Li 90026; Tahe, Li 90027; Silinji, Li 90029; Mudanjiang, Li 90035; Liuguo, Liu 90037

The seeds were put on a moist filter paper, and germinated at 22°C. When the roots grew and elongated about 1-2 cm long, the root tips were cut off and fixed in Carnoy's solution 3-24 h. In situ 4C DNA amount were measured in Feulgen cytophotodensitometric method. The fixed root tips of each species together with those of the standard plant were hydrolyzed for 12 min in 1M HCl at 60 °C, washed in distilled water and placed in Feulgen solution for 2 h at 23 °C. They were subsequently given three 10 min washes in SO₂ water (100 mL H₂O, 5 mL 1M HCl, 5 mL 10% (w/v) sodium metabisulphite). Each root-tip was squashed in 45% acetic acid. Nuclear DNA contents were estimated by microdensitometry in the method of Maxted et al. (1991) on Reichert-Jung scanning microdensitometer. An ex-

periment consisted of 3 slides of the standard plant, and 3 slides for each one of the test species. Only telophase nuclei were chosen for measurement. Ten nuclei were measured per slide and 30 nuclei were measured for all species and standards. The DNA content of test plants were obtained on the basis of absorbance, and expressed in pictograms using the known DNA content of the standard as calibration.

Results and Discussion

Cytotaxonomic relationship between sect. *Vicilla* and its allied section

Recently, Endo (1994) and Endo and Ohashi (1995, 1996 a, b) studied morphological features of *Vicia*, es-

pecially styles, stigmas and pollens, and proposed a new section, sect. *Amurense* Y Endo & H. Ohashi. This section includes *V. amurensis*, *V. dichroantha*, *V. num-*

mularia and *V. tibetica*. *V. amuresis* was a member of sect. *Vicilla* in Kupicha's system.

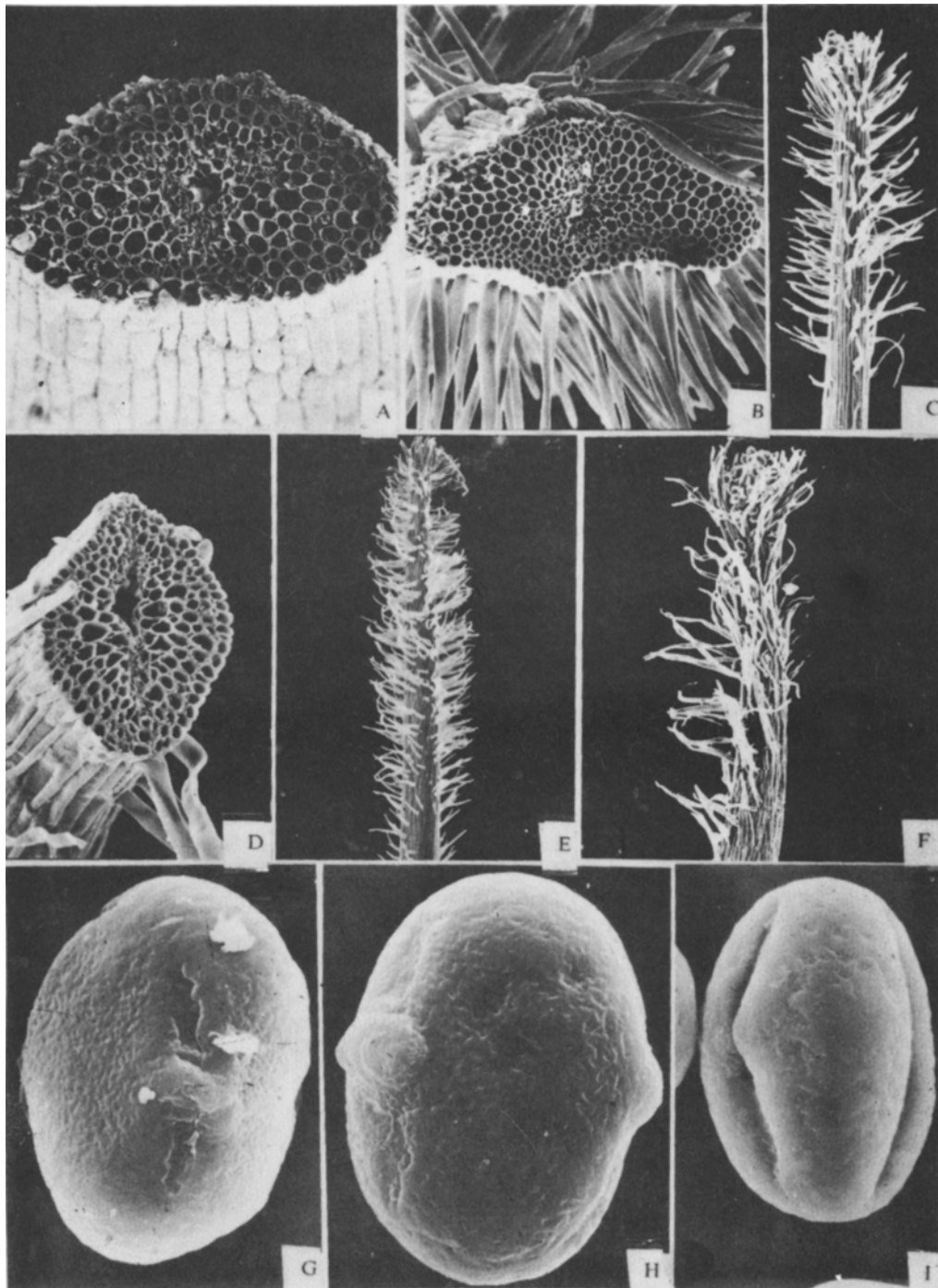


Fig. 1. Styles and pollen grains of three species of *Licia*.

Note: A. SEM phytograph of transverse view of style of *V. amoena*, X500; B. SEM phytograph of transverse view of style of *V. ramuliflora*, X350; C. SEM phytograph of lateral view of style of *V. amoena*, X300; D. SEM phytograph of transverse view of style of *V. amurensis*, X500; E. SEM phytograph of lateral view of style of *V. ramuliflora*, X70; F. SEM phytograph of pollen grain of *V. amoena*, X3.0 K; H. SEM phytograph of pollen grain of *V. ramuliflora*, X3.0 K; I. SEM phytograph of pollen grain of *V. amurensis*, X3.0 K.

Because those species have pollen grains with swollen mesocipium, terete styles, abaxially sprouting stylar hairs and globose stigmas, they differ from the others species in sect. *Vicilla* in Kupicha's system. We observe those morphological features of six species in *Vicia* which distribute in Northeast of China by means of SEM (Fig. 1, Table 2), and find that *V. amurensis* differs from

Chromosome number and morphology of *V. dichroantha*, *V. tibetica* and *V. nummularia* have not been reported. The somatic chromosome number of *V. amurensis* was found to be $2n=12$ (Nikiforova, 1984; Liu, 1988; Rudyka, 1986; Luo & Wang, 1989; and the present study). Rudyka (1986) observed presence of B chromosome in *V. amurensis*. In this study *V. amurensis* (Fig. 2, A) and *V. pseudo-orbus* (Fig. 2, B) (a member in sect. *Vicilla* of Kupicha's system) are diploid and have similar karyotypes. As they all have the same karyotype symmetry (2A), comparable chromosome size and the same type of Sat-chromosomes. Two types of Sat-chromosomes are found in the complements of those species: length of satellite is equal to one of short arm of Sat-chromosome in the first type, and in the second type length of satellite is longer than one of short arm of Sat-chromosome.

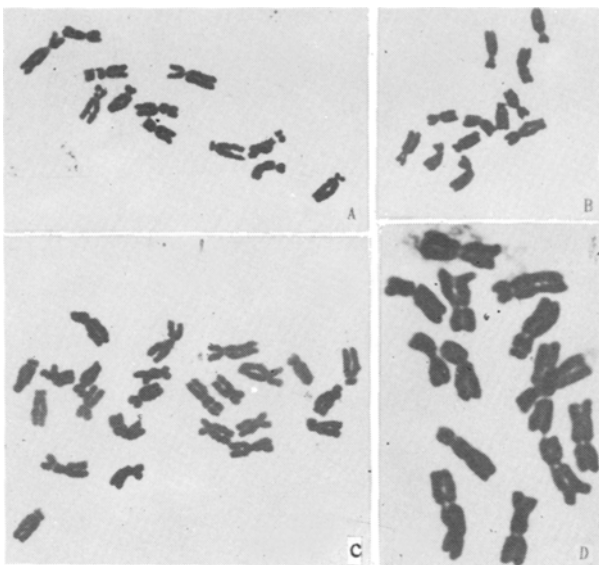


Fig. 2. Metaphase morphology of four *Vicia* species.

A. *V. amurensis*; B. *V. pseudo-orbus*; C. *V. japonica*; D. *V. dumetorum*.

However, they are different in some finer details. The first type of satellite-chromosome is respectively the 2nd pair of chromosomes in *V. amurensis* and 3rd pair of Sat-chromosomes in *V. pseudo-orbus*, and the 2nd type of Sat-chromosomes are respectively the 3rd pair in the

other five species in pollen, style and stigmas. Hence, species in sect. *Vicilla* of Kupicha's system could belong into two different sections:

Sect. *Amurensis*: *V. amurensis*

Sect. *Vicilla*: *V. amoena*, *V. japonica*, *V. pseudo-orbus*, *V. ramuliflora*, *V. unijuga* and *V. venosa*

former and 2nd pair of Sat-chromosome in the latter. Therefore, sect. *Amurensis* is closely related to sect. *Vicilla*.

Table 2. Features of style, stigma and pollen of sect. *Vicilla* and sect. *Amurensis* in Northeast China

Taxa	Stylar shapes ^a	Stigma shapes ^b	Pollen mesocipium ^c	Section
<i>V. amoena</i>	-	+	-	<i>Vicilla</i>
var. <i>oblongifolia</i>	+	+	-	<i>Vicilla</i>
var. <i>sericea</i>	+	+	-	<i>Vicilla</i>
<i>V. japonica</i>	+	+	-	<i>Vicilla</i>
<i>V. pseudo-orbus</i>	+	+	-	<i>Vicilla</i>
<i>V. ramuliflora</i>	+	+	-	<i>Vicilla</i>
<i>V. unijuga</i>	+	+	-	<i>Vicilla</i>
<i>V. venosa</i>	+	+	-	<i>Vicilla</i>
<i>V. amurensis</i>	-	-	+	<i>Amurensis</i>

Note: a: + dorsally compressed style, - terete style. b: + conical stigma, - globose stigma. c: + swollen mesocipium, - plain mesocipium.

Karyotype evolution of sect. *Vicilla*

Most researchers are known as that basic chromosome number ($x=7$) is a primitive basic chromosome number in the genus and the others are derived from it (Rousi, 1961). According to data of chromosomes reported in *Vicia* (Fedorov 1974; Goldblatt 1981, 1984, 1985, 1988, 1991). Distribution patterns of basic chromosome number in sect. *Vicilla* are differentiated between taxa in Europe and Asia. Most of European taxa have basic chromosome number of $x=7$, such as *V. dumetorum* and *V. sylvatica*. And taxa distributed in Asia are polytypic in basic chromosome number, having basic chromosome numbers of $x=5$, 6 or 7. Most of them are diploids at basic chromosome number of $x=6$, but intraspecific and interspecific tetraploids occur (Table 1). In addition, European taxa are different from Asian taxa in chromosome complement (Cincura 1973; Terziński 1974; the present study). Karyotypes of the former are consisted of metacentric and submetacentric chromosomes. For example, complements of *V. dumetorum* and *V. pisiformis* have four or five pairs of metacentric chromosomes respectively (Fig. 2, D). Karyotype of the latter is consisted of three types of chromosomes, i.e. metacentric, submetacentric and subtelocentric chromosomes. In those karyotypes, the number of metacentric chromo-

somes decreases obviously, and the number of sub-metacentric chromosomes increases, meantime one or two pairs of subtelocentric chromosomes are observed. In karyotype asymmetry, the karyotype in the section *Vicilla* is characterized by a moderate of asymmetry (Table 3). *V. japonica* has the highest asymmetry tendency (Fig. 2, C), while *V. pisiformis* has the smallest degree of asymmetry. The asymmetry index of *V. dumetorum* is 60.02, and one of *V. pisiformis* is 58.02. The asymmetry index of these two species is higher, which is due to their high number of metacentric chromosomes (four and five pairs) compared with the number of sub-metacentric chromosomes (two and three pairs). Therefore, the above results show that karyotypes of European taxa are more primitive and symmetric than that of

Asian taxa (Table 3). Also these results clearly show that a mechanism of increasing asymmetry degree probably be of decreasing number of metacentric chromosomes during speciation. Stebbins (1971) considered that species with more symmetric karyotype were specialized in morphological features. It is noticed that Asian taxa are polytypic in morphology. According to tendril and stem, those taxa can be divided into two kinds: plants without tendril and with eric stem, and plants with tendril and ascending stem. The former distributes in forest, the latter distributes in various habitats such as roadside, forest edge etc.. Hence, the present authors suggest that Asia probably be a center of modern diversification of sect. *Vicilla*, and Europe is an origin center of this section.

Table 3. Karyotype data of sect. *Vicilla* and sect. *Amurensis* in present study

Taxa	Formula	The longest/The shortest	Average arm ratio	As.I.
<i>V. amoena</i>	$2n=24=10m+10sm+4st$	1.60	2.09	66.49
var. <i>oblongifolia</i>	$2n=12=2m+8sm+2st$	1.41	2.61	66.95
var. <i>sericea</i>	$2n=12=2m+8sm+2st$	1.40	2.06	62.49
<i>V. bifolia</i>	$2n=12=4m(2SAT)+6sm+2st$	1.37	2.29	66.71
<i>V. dumetorum</i>	$2n=14=10m(2STA)+4sm$	1.49	1.57	60.02
<i>V. fauriei</i>	$2n=12=2m+8sm+2st$	1.27	2.48	68.42
<i>V. japonica</i>	$2n=24=8m(4SAT)+10sm+6st$	1.57	2.77	68.71
<i>V. pisiformis</i>	$2n=12=8m+4sm(2SAT)$	1.76	1.21	58.02
<i>V. pseudo-orbus</i>	$2n=12=2m(2SAT)+8sm(2SAT)+2st$	1.37	2.11	65.60
<i>V. ramuliflora</i>	$2n=24=8m+16sm(4SAT)$	1.68	1.94	64.49
	$2n=12=M(2SAT)+8sm+2st$	1.51	1.98	63.07
<i>V. unijuga</i>	$2n=24=8m(2SAT)+12sm+4st$	1.42	2.07	66.15
	$2n=12=2m(2SAT)+8sm+2st$	1.41	2.31	67.08
<i>V. venosa</i>	$2n=12=2M+2m+8sm$	1.32	1.98	64.47
<i>V. amurensis</i>	$2n=12=4m+2(SAT)+6sm(2SAT)+2st$	1.57	2.54	67.39

Variation of 2C nuclear DNA in *Vicia* sect. *Vicilla*

The DNA data obtained in the present investigation and Raina and Rees (1983) revealed significant differences in 2C DNA amount in *Vicia* sect. *Vicilla* (Fig. 3). Among diploid species, *V. dumetorum* has the highest DNA content (18.56 pg.), being 1.45 to 1.08 times more than the taxa in Asian. Another *V. pisiformis* distributed in European has similar DNA content to taxa of sect. *Vicilla* in Asia. *V. amoena* var. *sericea* has the lowest DNA content (12.75 pg.). Maxted et al. (1991) observed that the distribution of DNA amount in *Vicia* seemed to be independent taxonomic position. Decrease in chromosome number in the genus is accompanied by increasing taxonomic advancement. According to karyotype data, we observe that decrease of DNA amount among the studied species in sect. *Vicilla* are related with increasing taxonomic advancement. This differs from the results of Maxted et al. (1991) and Raina (1990).

In three intraspecific tetraploid cytotypes observed, variation are found between the highest 2C DNA amount for *V. unijuga* (32.45 pg.) and the lowest for two populations of *V. amoena* (18.60 pg. and 17.19 pg. respectively). Not only the variation in 2C DNA among genomes with the same ploidy level occurs, but also the tetraploid values are to some extent lower than the double conspecific diploid ones. Among three tetraploid cytotypes, *V. amoena* losses the highest 2C DNA amount, which is about 29.8 percent.

It has long has been known that in many groups the chromosomes in polyploids tend to be smaller than those in diploids. Two alternative explanations have been offered to explain why polyploid taxa may have less DNA per basic genome than the diploids. Darlington (1956) suggested that diploids with smaller chromosomes were preadapted to form polyploids. On the other hand, Grant (1987) considered that salutary changes

occurred in the genome and also this probably have happened at the diploid or polyploid level. Because intraspecific tetraploid cytotypes in *V. amoena*, *V. ramuliflora* and *V. unijuga* have less DNA amount per basic genome than the conspecific diploids, we suggest that the elimination of DNA has occurred in the polyploid cytotypes and not in their conspecific diploid cytotypes. Some authors considered that recent or ancient origin of polyploidy can be distinguished based on nuclear DNA amount (Bennet and Smith 1971; Poggio and Hunziker 1986; Palomino et al. 1995).

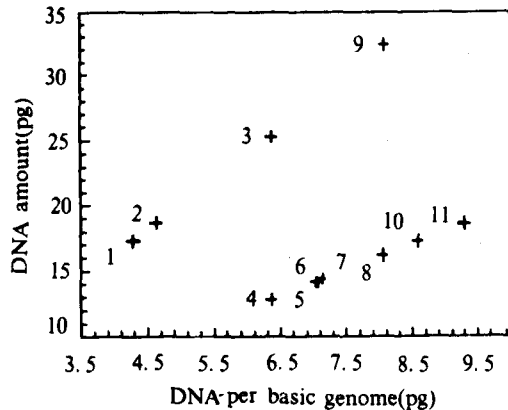


Fig. 3. Variation of DNA amount of *Vicia* sect *Vicilla* in Northeast China

Note: 1- Tahe population of *V. amoena*; 2- Qiqihaer population of *V. amoena*; 3- Changbaishan population of *V. ramuliflora*; 4- *V. amoena* var. *sericea*; 5- *V. pseudo-orbus*; 6- *V. pisiformis*; 7- Mudanjiang population of *V. ramuliflora*; 8- *V. sylvatica*; 9- Mudanjiang population of *V. unijuga*; 10- Jagedaqui population of *V. unijuga*; 11- *V. dumetorum*. Data on nuclear amounts *V. dumetorum* of and *V. sylvatica* obtained from Raina and Rees (1983).

The results in present study show that those intraspecific tetraploids could be ancient original. This suggestion may be supported by the distribution pattern of intraspecific tetraploid cytotypes.

Distribution patterns of intraspecific tetraploid cytotypes in sect. *Vicilla*

There is ploidy variation of chromosome number in four species in Northeast in China. Intraspecific polyploidy has been known in *Vicia amoena*, *V. unijuga*, *V. ramuliflora* and *V. japaona*.

Polyploid cytotypes are confined in a few sections (Table 4, data based on the following literatures: Fedorov, 1974; Goldblatt, 1981-1991; Hanelt and Mettin, 1989; Hsu et al., 1988; Li, 1993; Luo & Wang, 1989, and the present study). In sect. *Vicilla*, approximately

40% of all species has been observed to be polyploid cytotypes. Tetraploids and hexaploids are often indistinguishable from the conspecific diploids such as *V. unijuga*. Sometime, diploid cytotypes are different from conspecific tetraploid in their habitats and morphological features, for example, *V. amoena* complex and *V. ramuliflora*.

Table 4. Polyploids in the genus *Vicia* (data from Hanelt and Mettin in 1989 and the pre sent study)

Taxa	Chromosome numbers, $x=6$	Taxa	Chromosome numbers, $x=7$
<i>V. amoena</i> *	2x, 4x	<i>V. alpestris</i>	4x, 6x
<i>V. cracca</i>	2x, 4x	<i>V. americana</i>	2x, 4x, 6x
<i>V. edentata</i> *	4x	<i>V. cracca</i>	2x, 4x
<i>V. japonica</i> *	2x, 4x	<i>V. fedtschekdoana</i>	4x
<i>V. kulingiana</i> *	4x	<i>V. grossheimii</i>	4x
<i>V. macrantha</i>	4x	<i>V. magellanica</i>	4x
<i>V. nervata</i>	4x	<i>V. oreophila</i>	4x
<i>V. olchonensis</i>	4x	<i>V. villosa</i> spp. <i>varia</i>	2x, 4x
<i>V. ramuliflora</i> *	2x, 4x		
<i>V. unijuga</i> *	2x, 4x, 6x		
<i>V. woroschilowii</i>	4x		

Note: * showing taxa of sect. *Vicilla*

Diploids and tetraploids in sect. *Vicilla* show two types of pattern in geographical distribution: sympatric and allopatric pattern (Fig. 4 and Table 5). It has been reported that frequency of polyploid population increases from the south to the north temperate zone, and polyploid individuals are over their diploid ancestors in adapting to the extreme cold and draught. But the present study all is not in agreement with this point of view. In *V. amoena* complex, tetraploid *V. amoena* distributes widely in Northeast of China, but its diploid var. *sericea* restrictly distributes in special geographic area of Tongliao (the overlapping area among Liaoning province, Jilin province and Inner Mongolia), in which soil is sand. The another diploid var. *oblongifolia* is occasionally found and distributes in a unstable community together with tetraploid *V. amoena*. So *V. amoena* complex could be a mature polyploid complex. The diploid cytotype of *V. unijuga* is found in 50° north latitude, and its tetraploid one is in area between 46-40° north latitudes. The above result shows that diploid is distributed more northerly than conspecific tetraploid.

Table 5. Distribution patterns of intraspecific diploid and tetraploid in three species of sect. *Vicilla* in Northeast China

Taxa	Basic Chr. No.	Polyploidy	Diploid geographic	Tetraploid distribution	Sympatric between diploid tetraploid
<i>V. amoena</i>	6	4x	Tongliao Zhaodong	Northeast Zhaodong	- +
<i>V. ramuliflora</i>	6	4x	Northeast	Mts. Chang-baishan, 1600m Alt.	
<i>V. unijuga</i>	6	4x	Northern area of Northeast	Southern area of Northeast	-

In *V. ramuliflora* and *V. venosa*, tetraploid cytotypes have been found in China so far, but absent in Russia, Japan and Korea (Table 1; Nikiforova 1984; Endo & Ohashi 1988).

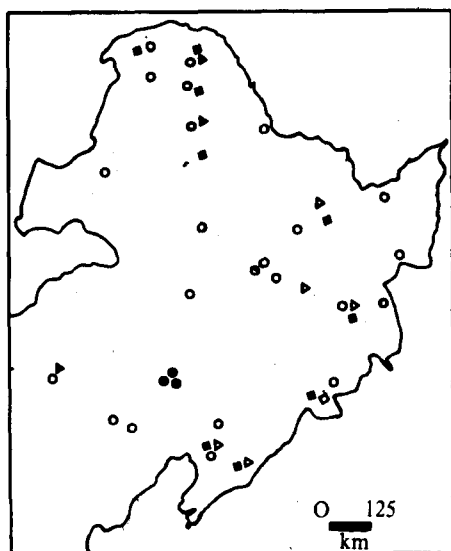


Fig. 4. General distribution of infraspecific tetraploids and conspecific diploids of three *Vicia* species.

Note: ○: showing tetraploid *Vicia amoena*; ◐: showing diploid var. *oblongifolia*; ●: showing diploid var. *sericea*; □: showing tetraploid cytotype of *V. ramuliflora* (Mts. Changbaishan, Alt. 1600-2000); ■: showing diploid cytotype of *V. ramuliflora*; △: showing tetraploid cytotype of *V. unijuga*; ▲: showing diploid cytotype of *V. unijuga*.

The two populations of cytotype exhibit disjunct distributions in China. One population is more restricted in distribution of Changbai mountains (area from Alt. 1600~2000 m) in Northeast in China, the other in Huangshan mountains in East China. The frequency of polyploid species tends to be relatively higher in the alpine area. Because those tetraploid cytotypes in *V. ramuliflora* are alpine perennial plants, their origin could be related with environment changes during the Tertiary period.

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